Office of Environmental Health Hazard Assessment

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Dr. Kenneth Olden
Director, National Toxicology Program
National Institute of Environmental Health Sciences
P.O. Box 12233
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Dear Dr. Olden:

I am writing to express my concern over the draft NTP Technical Reports on aspartame (GMM –01) and acesulfame potassium (GMM-02) in transgenic mice that will be peer reviewed at the upcoming Board of Scientific Counselors Technical Reports Review Subcommittee Meeting May 22, 2003. To treat these short-term studies conducted in incompletely understood transgenic mouse models as equivalent to the NTP's standard two-year carcinogenesis studies would be inappropriate and misleading. Because the transgenic studies of aspartame and acesulfame are being discussed in the same forum as standard two-year bioassays of four other chemicals, a clear distinction must be drawn concerning the limitations of the transgenic studies in comparison to the two-year bioassays with respect to the veracity and predictive value of the results. Furthermore, we are concerned that undeserved credibility would be placed upon transgenic short-term carcinogenicity models that have not, as yet, been adequately validated. We believe that because of the limitations of the transgenic models employed, the reporting of the results from these aspartame and acesulfame studies should be accompanied by a thorough discussion of the implications of a negative finding in these models and the possibility of false negative results.

In general, we believe that the transgenic models in most common use, p53 haploinsufficient, Tg.AC hemizygous, and RasH2, have not been sufficiently validated in comparison with two-year rodent bioassays and that further study is needed before they are given equal weight. While there is a good grasp of tests for genotoxic action, such as the series of *Salmonella* mutation assays and the interpretation of these assays are relatively straightforward, and while there is a great deal of experience and interpretation of two-year ("lifetime") rodent bioassays, at this time there is very little understanding of the various carcinogenic mechanisms operative in these transgenic mouse models. Moreover, p53 haploinsufficient transgenic

models, by virtue of their DNA damage response mechanism, are not expected to detect nongenotoxic compounds such as aspartame. The apparent inconsistency and insensitivity between transgenic and rodent lifetime bioassay results presently cannot be explained. The transgenic models may be useful for screening, as they can detect certain carcinogens, but negative results cannot be interpreted clearly. Thus, compounds giving negative results in transgenic assays must be further tested in two-year bioassays.

We support the plan to publish all transgenic studies in a new and separate NTP report series. We are troubled by the use of the term "carcinogenicity studies" in the titles of these first two GMM reports, to characterize the studies in the p53 haploinsufficient mouse model, and suggest that all studies in this and other transgenic models be referred to as "toxicity studies," the same term used in the titles of these two reports to characterize the studies in Tg.AC hemizygous mice.

We are troubled to see that the conclusions presented in these GMM reports mention only the findings in the p53 haploinsufficient mouse studies, using the same levels of evidence (i.e., clear, some, equivocal, no evidence and inadequate study) to characterize the carcinogenic activity of the test substance in this model as NTP employs when characterizing the results of a two-year bioassay, and are silent on the findings in the Tg.AC hemizygous mouse studies, and the genetic toxicology studies (*Salmonella*, rat bone marrow cells, and mouse peripheral blood erythrocytes). We suggest that the conclusion section of these GMM reports provide conclusions on all the studies presented in the report. We further suggest that each of these studies, including the transgenic mouse studies, be evaluated using descriptors more appropriate for these types of assays, namely, "positive," "negative," "equivocal" or "inadequate study."

In addition, we suggest that the new NTP report series include in the preface a discussion of what a positive, negative, equivocal, or inadequate study finding means, in terms of "evidence of carcinogenicity", "no evidence of carcinogenicity" or "not informative as to carcinogenicity". The finding that the transgenic mouse models tend to under predict carcinogens in comparison to the rodent lifetime bioassay should be discussed in terms of the overarching objective of protecting public health, and the role of animal testing in that endeavor.

In conclusion, while transgenic mouse models of carcinogenicity hold promise for the future, it is possible that their use should be restricted to screening protocols. Positive results in these screening assays do provide evidence of carcinogenicity. Given recognized limitations of these transgenic models; however, negative results are not informative as to the test substance's carcinogenicity, and point to the need to conduct standard two-year carcinogenicity studies. At this time, transgenic models cannot replace the two-year bioassay and it would be unwise to list a

chemical as safe for human exposure or consumption based upon negative results in not yet validated model systems.

Sincerely,

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